

4D and 5D Printing: Healthcare's New Edge

PRAMOD KUMAR, M.TECH • SUBARNA ROY • HARSHA HEGDE •
SHWETA BHARTI • MANISH KUMAR

INTRODUCTION

In the printing technology era the first patent was issued for the stereolithography (STL) apparatus, invented by Charles (Chuck) Hall in the 1980s. Till the 20th century the printing technology was very limited and people used to write on paper, which is known as (two-dimensional [2D]) printing technology. The concept of three-dimensional (3D) printing of object was introduced by Charles W. Hull in 1984, a concept in the manufacturing world: direct printing of objects in three dimensions.^{1–3} Initially this technology was known as rapid prototyping and with further advancement, it is now known as additive manufacturing or 3D printing technology. 3D printing technology is implemented not only in several industries for production but also in medical fields, hospitals, school, households, and offices.

3D printing methods refer to fabrication of biological and medical objects with bioink composed of biomaterials and living cells (Murphy et al., 2014).^{4–6} The shape deformation may perform unique functions using biological systems. The central nervous system and the neurotransmitters susceptible to stretchable muscle tissues play a significant role in regulating movements in mammals. The static behavior after printing and shortage of stimulus responses to 3D bioprinted objects inhibit structural and functional deformation to perform any function like native bio-objects. An innovation in printing technology is four-dimensional (4D) printing. 4D printing was first introduced in 2013 and immediately spurred great attention in various research areas including, but not limited to, smart materials and biomedical research.^{7–10} Mostly 4D structures are developed by incorporating shape transformation within the material/structural design that contributes to the 4D printing definition: 3D printing of objects that can, immediately after printing, self-transform in form or function when exposed to a predetermined stimulus,

including osmotic pressure, exposure to heat, current, ultraviolet (UV) light, or other energy sources.^{8,9,11,12} 4D printing technology is able to print 3D objects derived in a predefined manner in a certain period under the influence of external stimuli. 3D printing technology not only has the potential to evolve in terms of hardware but also has the capability to play a key role in the progressive development of new materials, with enormous potential for various applications. 4D printing technology is based on unique feature development, whereby printed objects can be accelerated to further alter their shape and size in a certain period. To achieve this objective, the printed material should be capable of responding to various stimuli such as heat, pH, light, and magnetic field. Under the certain conditions such as shape deformation or change in function and influence of external stimuli, 3D objects can be fabricated by using smart materials. Such stimulus-responsive 3D printed objects of smart materials are termed as 4D printed objects.

4D AND 5D BIOPRINTING TECHNOLOGY

4D Printing Technology

The progress of 3D printing technology in soft materials is being used in several medical fields, with the progressive development of an innovative technology called 4D printing.¹³ In other words, 3D printing technology has progressed to another printing additive manufacturing technology known as 4D printing. The conceptual theory of 4D printing involves “time” as the fourth dimension in the 3D space coordinates.¹⁴ In 4D printing technology system, 3D printed objects that have not taken their final structure at the time of printing are allowed to change their form over the time interval in response to external resource of stimulation, such as temperature, light, and electricity.^{12,15,16} Hence, 4D printing technology is being considered as a novel

cutting-edge technology evolving from 3D printing, smart materials, and programming.

In this emerging and novel technology, smart materials have an important role, which are capable of being flexible, expandable, and deformable under an applied stimulus.¹⁴ However, only a few smart materials are able to be 4D printed, even though much research to develop new materials has been accomplished. The 4D printable materials contain unique properties, such as temperature-responsive polymers,¹⁷ photoresponsive polymers,^{15,18} and ionic electroactive polymers.¹⁹ When the electric circuit design on a 2D structure and the self-deformation mechanism by considering material properties to achieve 3D circuit designs are combined, the 4D printing technique would provide much faster and cost-effective methods for the fabrication of smart devices embedding 3D circuits.

The term 4D printing was identified and developed by the collaboration of two institutions: Massachusetts Institute of Technology (MIT) Self-Assembly Lab and Stratasys Education and R&D department. In February 2013, Skylar Tibbitts, codirector and founder of the Self-Assembly Lab located at MIT's International Design Center, unveiled the technology "4D printing" at the TED conference held in Long Beach, California.⁹ 4D technology is still in the early phase of research and development. This technology is being used in several laboratories and prototyping facilities. At present, one cannot just order and buy a "4D printer." As of 2017, MIT's Self-Assembly Lab, the 3D printer manufacturer Stratasys, and the 3D software design company Autodesk are the key players in the development of 4D printing technology.

What Is 5D Printing?

The first-ever 5D printing technology was introduced about 2 years ago by American universities but this technology was implemented by the Mitsubishi Electric Research Laboratories (MERL). According to Dr. William Verazunis, "A 5D printer allows for objects to not be printed from one point upwards but from five axes. Hence, where the number five in 5D comes from. The print head moves around from 5 different angles while printing because the plateau on which the object gets printed moves as well. These movements allow the printer head to come in from many different angles, otherwise not achieved with 3D printing. These new angles result in the printing head being able to follow the path of the object's shape and outline. By not having to follow a straight path on a static plateau – and using the shape of the object instead, the printed parts

can be created with curved layers instead of flat layers. These curved layers allow for stronger parts that have a complex design to be printed."

How Can 5D Printing Be Applied?

The feature of 5D printing is an amalgamation of subtractive and accompaniment techniques. As per MERL, 5D printing technology requires some beforehand analytic analysis on how the 5D printed parts will be used. Therefore the best way of implementing 5D printing is for complex structures and designs, which require a lot of strength. We can think of 5D printing, for example, a concave cap, an item that could not be 3D printed because it needs a lot of fillers and support, as well as its design is too complex.

Differences Between 3D, 4D, and 5D Printing

The difference between 3D and 5D printing is that 5D printing depends on a moving plateau that allows for the printhead to make different angles from five dimensions and create curved layers, whereas 3D printers create flat layers on a fixed plateau. The remaining process is the same for both processes in general. Both processes make use of a 3D scanner and the same kind of 3D design, 3D file, and 3D printing materials.

4D printing technology is unique and very different among all the three printing technologies because it depends of various different smart materials and 3D models. Programmable materials are exposed in 4D printing; these materials change their function when exposed to hot water, light, or heat. Smart materials use materials with thermomechanical properties and other material properties as input, which allow for shape changes, and are different from the common 3D printing materials.

Smart Materials

The global competition among technologic giants and the demand for a new generation of industrial, commercial, medical, automotive, and aerospace applications have fueled research and studies focused on advanced materials and smart structures. Scientific researchers are trying to develop smart and ultimate materials that can be used in developing multipurpose scientific and technologic applications. These smart materials have some specific features, such as fibrous polymeric composite materials capable of sensing external stimuli in the form of heat, light, electricity, magnetic field, water, and several other agents. Distinct applications and different structures of smart or intelligent materials have

revolutionized the present generation. If we look back at the history of materials, starting from wooden and stone materials to the Stone Age to copper followed by bronze and Iron Age, humans have developed and set a new age, which we can call as the Smart Material Age.

Humans are directly influenced by materials technologies and those materials have made us remarkable among other living beings on this planet. The novel and current fabricated materials featuring plastics, composites, and biomaterials represent the new age of materials. Several innovations in various scientific fields, including manufacturing, nanotechnology, material science, and automation featuring as intelligent or smart materials, are capable of having a positive impact on civilization. The people of 20th generation are familiar with some classes of materials that show some autonomous existing functions in response to changing environmental stimuli, which are embedded sensory capabilities in order to comply with the programmed shape.

Smart materials assimilate with sensors and actuators that are highly integrated within the structure functionality. Some of the features such as signal conditioning, signal power amplification, and highly integrated control logic in a material are enhanced by a mechanical, thermal, optical, magnetic, or electric source (Table 8.1). Smart materials associated with a light source are able to change their color and shape, and mechanically smart materials undergo alterations in their mechanical states such as position, velocity, stiffness, and damping. The transition of laminated materials technology, made by smaller constitutive

elements, helps to expand the active element within the structure. Smart ply or composite materials could be developed with actuators, sensors, processors, and interconnections. The progressive advancement of microelectronics, switching circuitry, fiber-optic technology, and information processing techniques has further influenced the evolution of smart materials. Table 8.1 represents²¹ the classification of smart materials,²² along with the input force they require and the output.

4D PRINTING METHOD

When 4D printing technology was introduced by Skylar Tibbits in 2013, he demonstrated a structure folded with only 90° transformation and activated when the printed specimen was buried in water. Similarly research has demonstrated the composite printed materials that stretch upon heat activation, the light-activated (LA) materials, and the electrically activated materials. As progressive development is going on, still some more development is needed in folding from one shape to another. Also, some improvement is needed on taking control over autonomous transformation rather than using a human-guided energy source.

A challenge in 4D printing technology is the design structure, including both the software and hardware sections. To design the hardware part, special features need to be addressed. This requires specific features such as complex and advanced material programming, precise multimaterial printing, and designing complex joints for folding, expansion, contraction, curling, and twisting process. Designing the software section is more challenging, which cooperates with the hardware design. Some of the major challenges, including the sophisticated simulation, material optimization, and topology transformation, are present in the software part. The following sections about structural transformation, regarding its joint angle, folding, curling, and bending.²³

Fabrication

The printer collects UV-curable polymer and presents each layer using UV light, thereby creating a complete 3D structure; printers print multiple composite materials with different properties such as color pattern, material hardness, and transparency, allowing the creation of complex, multiple composite parts in a single process. Digital materials could be printed using this process. This type of properties could be digitally adjusted and modified with the digital material. The amalgamation of digital material, along with the different proportional and structural arrangements, plays a symbolic role in providing additional flexibility. 4D

TABLE 8.1
Classification of Smart Materials

Types	Input	Output
Piezoelectric	Mechanical stress	Potential difference
Electrostrictive	Electric field	Deformation
Magnetostrictive	Magnetic field	Deformation
Thermoelectric	Thermal energy	Deformation
Shape memory alloys	Thermal energy	Deformation
Photochromic	Radiation	Color change
Thermochromic	Thermal energy	Color change

printed parts usually contain rigid plastic and digital material that reacts with an external energy source. When a hydrophilic UV-curable polymer is exposed to water, it absorbs water and forms hydrogel, with up to 150% of the original volume. In this case the linear structure exists, but when the polymer combines with a composite material, it reacts differently with water and a complex geometric transformation takes place. The transformation could be controlled by using pure expandable polymers with a digital composite material as per requirement.²³

Joint and Folding Angle Strands

In any type of bending structure, joints play a key role because controlling of the joints adjusts the desired shape of the structure.

4D printing technology joints contain multiple layers of material. The composition of an expanding material, a rigid polymer, and a digital material depicts the folding direction and pattern. Those materials are likely to be arranged above or below each other as per the type of transformation. If an expanding composite is present above a rigid polymer, the surface would fold downward and if we try to move it below, the surface would fold upward. This process of folding happens due to the downward or upward force applied to the rigid material. Using a digital polymer composite makes the control of folding the joints much easier and desirable. The time required for folding depends on the expandable material or the digital material. If a higher expanding composite is used, the folding forces may be more, thus increasing the folding time. Similarly a less expanding composite would generate less folding force, thereby decreasing the folding time. The angle could be controlled by printing rigid discs at each vertex. The distance between the rigid discs and the diameter of the discs show angular variation. Discs bump with each other when the expanding material is activated and stop folding at a certain angle. The angle of folding is determined by the distance between the rigid discs and their diameter. When the discs are placed farther away from each other, the angle of folding will increase and when the discs are placed close to each other, the folding angle decreases. Also, the increasing diameter of discs decreases the folding angle and vice versa (Fig. 8.1).²⁴

Custom Angle Surfaces

During his research, Skyler Tibbits has demonstrated about how custom angles created angle transformation from a truncated octahedron shape. Similar to the mechanism of folding strand, demonstrated previously,

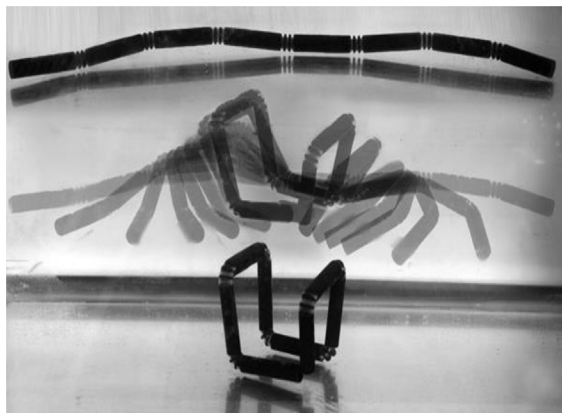


FIG. 8.1 Self-folding strand.²⁴

the series of flat 2D structures were described with edge joints. The folding angle and position are fixed by the position and spacing of materials at each joint. The folding accuracy may be monitored using codes producing custom angle joints and including the dates from calibration test. After the digital model was sent to be printed, the physical model was immersed in water. The transformation phase may occur within a certain time, with the edges perfectly aligned with the neighboring edges in the final predicted model. As per this technique, a 2D polyhedron was folded and self-transformed into a precise 3D structure.

The major advantage of this technology is that minimal resources and less time are required for flat shape printing. The final model, however, takes comparatively longer time and requires more support materials. This technology has several benefits in the long run, where flat surface materials could be created, shipped, and self-transformed into 3D structures (Fig. 8.2).²⁴

Curved Surface Folding

The curved folding technology depends on a new technique called curved-crease origami, where 2D flat sheets could be folded along with curved creases to form a double curved surface along with mountain- and valley-shaped linear pattern.²⁵ This mechanism could be explained with the example of concentric circles containing expanding polymers separated by rigid or less expanding polymers. The above or below position in each circle depends on the rigid polymer in each circle, with the ring being neutral, creating mountain and valley folds. If we place the design part in the water, after a particular time interval, the structure self-transforms from a 2D crease to a doubly curved structure (Fig. 8.3).²⁶

Surface Curling

Instead of joints, edges, and curved-crease folding, the same mechanism could be applied to surface curling. The continuous surface can be achieved by using



FIG. 8.2 Self-folding truncated octahedron.²⁴

a gradient of material deposition. Rather than using expanding materials as in the joints and folding process, larger expanding polymers could be used to have an even expansion force. The curling effect could be more visible with increasing length and breadth of the surface. If we try to place the expanding material above the rigid material, a curved surface with smaller radius is formed, and similarly if the expanding material is placed below the rigid material, the reverse effect can be seen. If joints and folding process does not take place in folding process, the quantity of material distribution would be important for maximum precision.²³

5D PRINTING

The revolution in printing technology, i.e., 3D printing known as additive printing, is now under extinction. One of the emerging innovative and widely used technologies is now under progress, with 4D printing being challenged. MERL has just opened the gate to 5D printing.

Before starting to digest this complexity, recapping about 3D printing will be the first step in understanding what it is all about and what 4D printing would have been to finally introduce 5D printing. 3D printing refers to the process of producing a 3D object. Using a 3D printer the desired object is computer-scanned and molded into a polygon it needs to be. Jeremy Rifkin, a futurologist, social theorist, and the author of *The Zero Marginal Cost Society*, crowned the 3D printing's era as the beginning of a new industrial revolution, as it is



FIG. 8.3 Curved-crease origami.²⁴

able to replace traditional manufacturing with maximum production and by eliminating a large amount of time to produce such objects. 3D printing also evolved into 3D bioprinting, wherein it was successfully able to print usable human organs for patients who need an organ transplant. 3D printing has been the king of all successful industrial technology that stood today. 4D printing, however, theoretically will be able to produce industrial objects that move and morph to the next possible angle independently via a computer program. This ability should enable the 4D printed object to potentially adapt to the environment it is exposed to. This high-tech definition of 4D printing made its way up to 15–20 years of research and development that did not intimidate 3D printing.

Now MERL made the initiative to step into the future and potentially take 3D printing to new heights. 5D printing is not really the next level of 4D printing. 5D printing stands for 3D printing with five axes of 3D printing. This means that it disappointingly does not do more than 3D printing but makes additive printing much stronger because it uses five axes of additive printing, meaning the foundation of the 3D printed object is not flat layers anymore but a series of round solid formations. This allows the object to be five times stronger than the traditional 3D printed object and less amount of material is needed to build.

An experiment was designed by the principal scientist of MERL, William Yerazunis. The experiment determined the strongest 3D printed material. There are two pressure caps that are 3D printed and each is exposed to a pressurizer. One is made by the traditional 3D printer and the other is made by the MERL 5D printer, and after the objects were tested, the MERL 5D printed pressure cap was found to be successful (Fig. 8.4). The 3D printed pressure cap broke easily under just 0.1 MPa

pressure, whereas the 5D printed pressure cap lasted up to 3.7 MPa. The goal of MERL is to produce products that are durable in areas where durability is needed.

4D AND 5D PRINTING IN MEDICAL RESEARCH

A 3D printed stint that gets absorbed into the body over time was developed by the University of Michigan. For the patient who was having weak cartilage in the walls of bronchial tubes, the stint was used to open the airways for 2 or 3 years, within this duration the bronchial cartilage came back to its shape. These 3D biomedical splints change shape and conforms over time with the body as it moves or grows. Different implants of 4D printed structures were developed, which need to be biocompatible with the patient's immune system and are able to adapt to the surrounding tissues in the human body. The technique started with a virtual model of trachea using a CT scan from the patient and framing a model of the virtual stint with a medical imaging software called Mimics. A biomaterial named photocleavable linker (PCL) was used to print the stint using the Formiga P100 3D printer.²⁸

In the near future, 4D printing technology would be used for all kinds of implants and reconstructive surgery. Patients with respiratory issues can also be benefited; researchers are exploring its use to correct human skeletal deformation such as facial rebuilding and ear reconstruction.

The human body's complex structure could be 3D printed, which can be helpful for problems with creating organs. 4D bioprinting can also play a key role in creating mature 3D printed tissues, which can function similar to natural tissues and organs. 4D bioprinting is a challenging and emerging technique for medical applications such as in tissue engineering and drug delivery, but it is still in its progressive stage. The main role of 4D printing technology is stimulus, which can respond and activate the printed object. In 4D bioprinting technology, biomaterials get mature enough in response to internal stimuli.

Among the many biomedical applications in the medical field, tissue engineering helps to design and fabricate naturally mimicking tissues and organs, which can be placed instead of damaged human organs.

Additive manufacturing suppresses solid scaffold tissue engineering based on simultaneous 3D positioning of cells with higher density, which allows vascularization of thick tissues. The maturation of tissues takes place based on high-density cells. The 3D positioning of different cells in space also creates vascularized

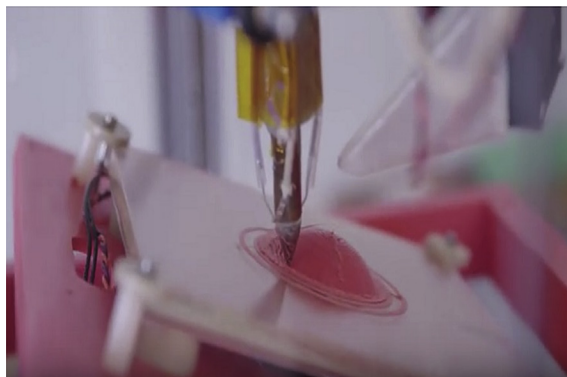


FIG. 8.4 5D printed pressure cap.²⁷

organ printing.²⁹ In biomimetic vascular systems, the vascular tissues can be fabricated using self-folded polymers. Additive manufacturing is also used to print scaffold-free mini tissues by using bioinks of different sizes and shapes to fabricate larger tissues and complex organs (Fig. 8.5).³⁰ In the human body, muscles play a significant role in regular activities. Calcium ions are important for muscular tissue movement. Muscle-like movement in printed tissues is achieved using ion-responsive biocompatible materials.³¹ These ion-responsive tissues show promising effects in regenerative medicines.

Rather than tissue engineering, 4D bioprinting has a significant impact on drug delivery applications. 4D printing technology performs targeted drug delivery in a controlled manner. Anticancer drugs have low tumor-targeting efficiency, which affects both tumor cells and the normal cells. 4D bioprinting technology facilitates precise drug delivery at the target site of tumor cells in a programmed manner. 4D printed pH-responsive and magnetic field-responsive soft microrobots of a hydrogel bilayer structure contains iron oxide particles (Fe_3O_4) that move to the target site at $600\ \mu\text{m s}^{-1}$ in an external magnetic field and unfold at pH 2.6 to release an encapsulated anticancer drug in a controlled manner.³² These thermomagnetic soft microgrippers can be used in surgery. Magnetically designed and derived

bio-objects are used to develop movable organs such as human fingers (Fig. 8.6).³³ Similarly, pH-responsive layered hydrogels, bilayers containing different hydrogels of different swelling ratios, can also be used for encapsulation of drugs and targeted drug delivery.

There are several biomedical devices being developed and they have been used for 4D bioprinting for use in medical and healthcare applications. Researchers discovered and developed artificial valves made of hydrogels, inspired by the water and gas regulator of plants, namely, the stomatal complexes present in the lower epidermis of plant leaves and stems (Fig. 8.7).³⁴ This hydrogel valve is responsive to external stimuli. These types of technologies can be used for drug delivery and blood circulation applications. Another device has been developed for treating life-threatening tracheobronchomalacia.³⁵ The device has been designed by using bioresorbable PCL and this can change the geometry of airways during respiration.

BIOMATERIALS FOR 4D AND 5D BIOPRINTING IN MEDICINE

In the below section, 4D printing–integration of transforming data in structural design, humidity-sensitive, water-sensitive, thermoresponsive, and natural solvent-sensitive materials have been discussed and highlighted

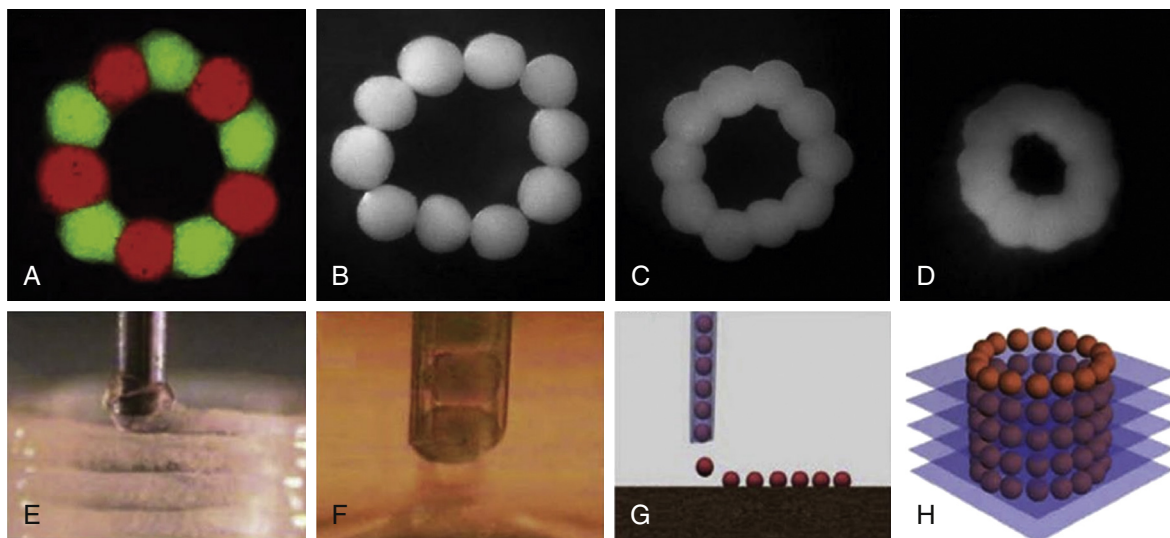


FIG. 8.5 Self-assembled ringlike vascular tissues for the construction of spheroid tissues fabricated from human smooth muscle cells. **(A)** Spheroid tissues are labeled with *green and red fluorescent stains* to demonstrate the absence of cell mixing during tissue fusion. **(B–D)** Sequential steps in the morphologic evolution of a ringlike vascular tissue construct during tissue fusion. **(E)** Bioprinting process. **(F)** Spheroid tissues during printing. **(G,H)** Schemes of layer-by-layer deposition process.³⁰

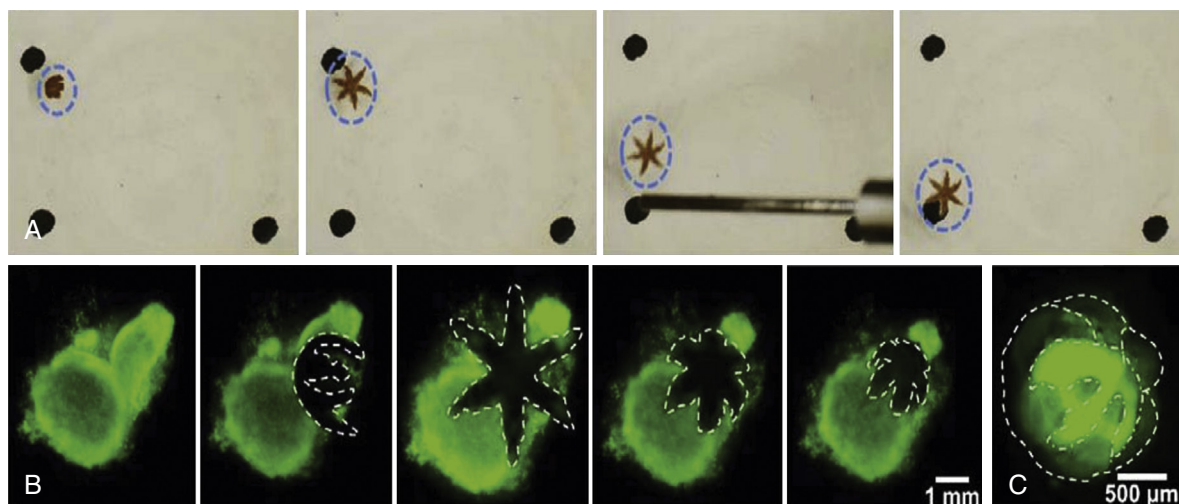


FIG. 8.6 Applications of the soft thermoresponsive self-folding grippers. **(A)** Video snapshots showing the guidance of Fe_2O_3 -doped polymer grippers between two round black marks using a magnetic probe. **(B)** Capture and excision of cells from a live cell fibroblast clump. **(C)** Gripper with excised cells within its grasp. The dashed lines are added to aid visualization of the gripper and the excised cells.³³

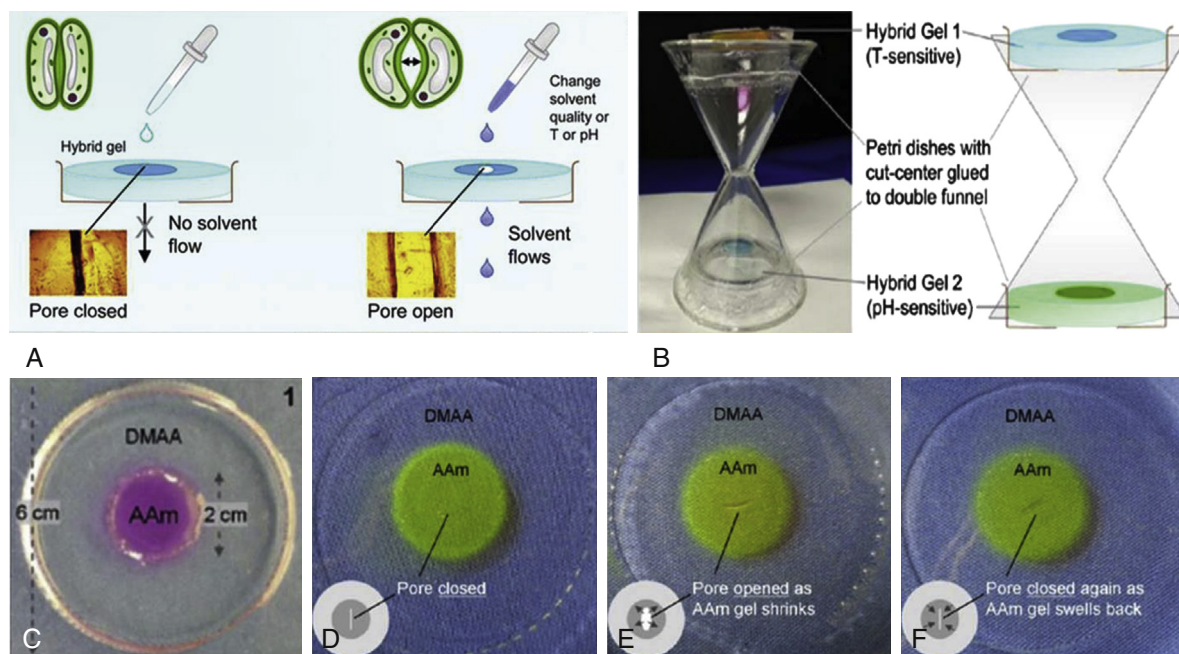


FIG. 8.7 **(A)** Hybrid hydrogel valve system inspired by the stomata in plants. **(B)** Dual stimuli-responsive hydrogel valve system. **(C)** A typical hybrid hydrogel system. **(D–F)** Hydrogels in response to solvents. The pore was initially closed in water and opened in 60% acetone system and again closed back in water.³⁴ AAm, acrylamide; DMAA, dimethylaminoethyl methacrylate; T, temperature.

for 4D bioprinting processes. Besides these terrific functional materials, a myriad of stimulus-responsive systems and novel approaches also exhibit probable potential for 4D printing technology.

Thermally Induced Novel Processes

The interior strain/stress during fused deposition modeling (FDM) 3D printing has been used in patterned transformation(s).³⁶ In FDM printing technology, polylactic acid (PLA) filaments usually placed in a furnace at a high temperature and then extruded through a nozzle. Temperature differences exist between the printing chamber and furnace and the extruded material moderately cools and solidifies, thereby bonding to the printing platform and previously deposited materials. The created internal stress/strain of the material

progressively increases during the printing process as temperature differences occur and attraction force is induced through a feeder motor. When the existing temperature goes below the glass-transition temperature (T_g) of the material, the stress/strain could be stored for an extended period. Release of the internal stress/strain can be achieved by heating the printed architecture above the T_g , which triggers shrinkage or pattern transformation. Fig. 8.8A emphasizes the transforming mechanism from a ring structure to quadrangles.³⁶ The shrinkage process in the printed structure occurs at high temperatures, which shows the unwanted effect of FDM, but the previous design can be achieved by operating the internal stress/strain in a controlled dynamic process. In multilayer membrane structures of different kinds of materials, within each and individual layer a

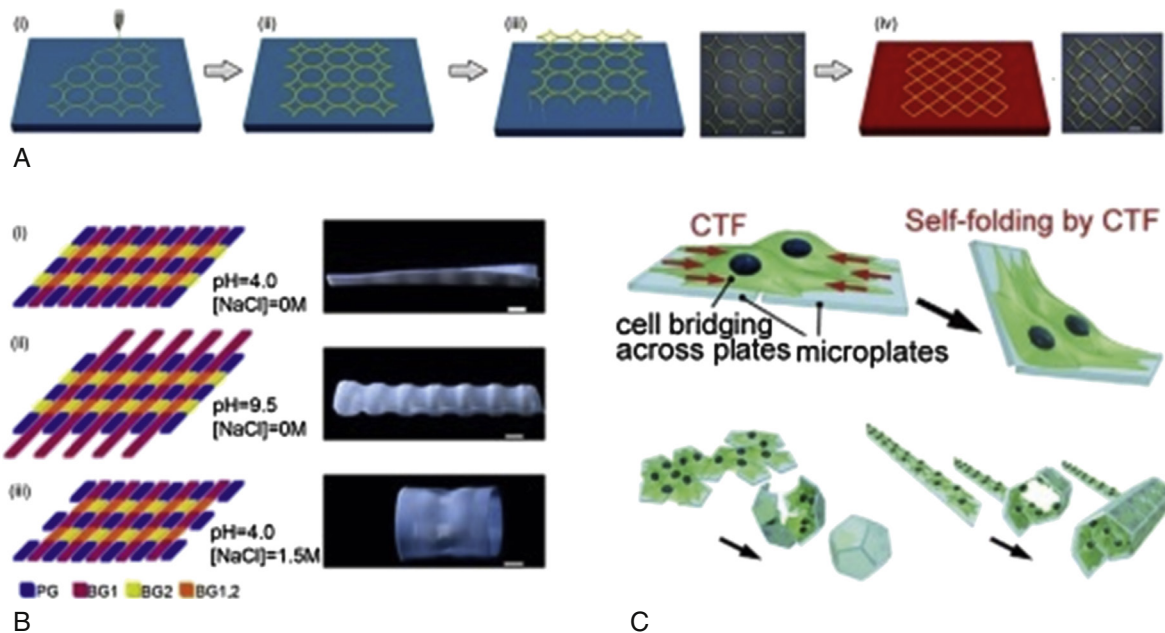


FIG. 8.8 Novel processes and materials with great potential for 4D printing. **(A)** Schematic of 3D printing process and the corresponding deformation of a printed polymer described by a viscoelastic model: (i) the fused polymer is extruded from the nozzle and a constant strain is formed because of the moving of the nozzle; (ii) the printed polymer cools, solidifies, and bonds with the platform or neighboring material and internal strain is generated during the process; (iii) removal of the printed polymer from the platform; and (iv) internal strain stored in the polymer is released when reheated above its glass-transition temperature. Scale bar is 12 mm. **(B)** Multiple shape transformations of the composite gel sheets. The scale bars are 0.5 cm. **(C)** Schematic image of the cell origami: the cells are cultured on microfabricated parylene microplates, which are self-folded by cell traction force (CTF). (Adapted with permission from Kuribayashi-Shigetomi K, Onoe H, Takeuchi S. Cell origami: self-folding of three-dimensional cell-laden microstructures driven by cell traction force. *PLoS One*. 2012;7(12):e51085. Thérien-Au H, Wu ZL, Nie Z, Kumacheva E. Multiple shape transformations of composite hydrogel sheets. *J Am Chem Soc*. 2013;135(12):4834–4839. Zhang Q, et al. Pattern transformation of heat-shrinkable polymer by three-dimensional (3D) printing technique. *Sci Rep*. 2015;5:8936.)

heat-stimulated dynamic process can be generated, owing to the mismatching coefficients of thermal expansion between materials.³⁷ The flat PLA strips printed on a fixed paper and later on a composite sheet are cut into the desired shape with six petals and kept on a heating plate at 105°C, with the PLA layer facing upward; the center of the composite sheet rises at the beginning and falls steadily to flatten while increasing the temperature and then it reaches equilibrium.³⁷ When the temperature decreases gradually and reaches a cooling point, the composite is transformed into a flowerlike 3D structure, with the incurvation of PLA inside and paper sheet outside; when it gets reheated the flower structure turned out to its original planar shape.³⁷ In addition to heat, light is able to reversibly induce conformation changes of certain materials.^{32,38,39} A smart box with decreased graphene oxide-carbon nanotubes/polydimethylsiloxane actuators as the hinges will unfold under simulated sunlight irradiation and will fold when the light is removed.³⁸ A laminated structure was constructed for achieving photoinduced deformation.³⁹ The laminated structure contains three bonded layers. The electrostatic sheets have been printed with two light-active polymer layers. If one side of the light-active polymer layer was radiated to be optically relaxed, the stress in the middle elastomeric sheet was released, triggering bending deformation. Self-healing polymeric materials have been developed for designing 3D biomimetic tissues such as skin.⁴⁰ Especially, when hydrogel materials exhibit a 3D network structure and magnificent water-retention abilities, they provide conditions related to those of the extracellular matrix (ECM).⁴⁰ Therefore soft hydrogels are considered as the ideal materials for use in tissue engineering applications.^{41–43} Hydrogels can be used in several commercialized products such as wound dressings, contact lenses, and personal hygiene products.⁴⁴ Hydrogels contain active compounds, such as drugs and antibodies, which have highly active research area, and hydrogels are the key product for the development of artificial implants.^{45–47} Interestingly, self-healing hydrogels can automatically heal damaged tissues and restore normalcy; this ability seems to be a dynamic process identical to 4D printing and it motivates the exploration of self-healing hydrogels in the field of organ regeneration and tissue engineering.⁴⁰ The self-healing process takes place as the hydrogel forms new bond, with electrostatic attraction forces, using reconstructive covalent dangling side chains or noncovalent hydrogen bonding.⁴⁰ So far, 4D printing of self-healing hydrogel materials have not been reported; therefore, a myriad of hydrogel materials for 3D/4D printing are observed, predicted, and reviewed.⁴⁰ The integrated multiple

transformations are brought out by different stimuli as a “single” shape, which catalyzed the smart material field; several studies have been conducted on the development of materials and structures that were configured and reconfigured several times using different stimuli, which are covertly revolutionizing related fields.^{48,49} Multiple shape transformations, each provoked by a particular external stimulus, were accomplished with a planar hydrogel sheet combined with multiple small-scale polymer components with distinct compositions.⁴⁸ As internal stress exists within composite hydrogel sheet, structural components undergo differential shrinkage or swelling in response to different stimuli (i.e., temperature, pH, ionic strength, or CO₂ supply Fig. 8.8B).⁴⁸ The complicated microenvironments in the human body are physiological analogues to this concept and contain multiple regulatory processes, such as neuroregulation, self-regulation, and humoral regulation, that take place when the complicated microenvironments in the human body are physiological similarly to this concept. Therefore multiresponsive materials can be a better option for biomedical applications. Discovering and identifying novel multiresponsive materials, or evaluating and transforming the printability and biocompatibility of existing multiresponsive materials, may become an exciting research trend.

APPLICATIONS AND EXAMPLES OF 4D PRINTING IN HEALTHCARE

Applications of 4D Printing in Tissue and Organ Regeneration

3D printing has always given its best to engineer functional organs and tissues. It does so by healing or replacing nonfunctional organs and tissues and in turn provides an effective solution to overcome the shortage of organ/tissues.^{4,51} 3D printing is evolving rapidly and seems to ace in the field of biomanufacturing technology.^{52–61} By picking out the advanced features of 3D printing technology, 4D printing houses a time-dependent process in fabrication design. This is believed to revolutionize the existing organ/tissue fabricating platforms. Synthetic tissues that can be folded and can also be controlled by light were turned into structures containing hundreds or thousands of communicating aqueous droplets arranged in programmed patterns.^{62,63} The process begins by constructing a piezo-based 3D printer that is capable of printing thousands of aqueous droplets (50 pL) in patterned 3D assemblies. The printed droplet networks were generated inside oil drops in an aqueous environment, thus the final structure is encased in a

lipid bilayer through which communication with the external environment can be mediated using membrane proteins.^{63,64}

These structures have the ability to perform dynamic shape change processes. Droplets in the form of layers, which have different internal osmolarities, were printed as four-petal structure. This structure folded into a hollow sphere where water flowed through the bilayers (Fig. 8.9A). These structures, which have multiple communicating compartments and also have the ability to change shape dynamically, are rendered analogous to living organisms. In addition, these structures can also be enhanced so that they resemble living cells by making them to synthesize protein from encapsulated DNA with an *in vitro* expression system, which can be controlled by external light.⁶² Fig. 8.9B shows the light controlling expression system.

Biotin is attached at some sites on the promoter region of the gene of interest. Streptavidin binds strongly with biotin, used as a steric blocker of the promoter sequence. This prevents the transcription of the DNA by RNA polymerase. Additionally, linkers such as the PCLs were also inserted between the biotins and the promoter. Thus the expression system existed in off-state at first; after removal of the block with low-energy UV light, transcription occurred, followed by translation of the messenger RNA into protein. Optimizing the number of biotins and their sites of attachment led to production of the tightly regulated, LA- DNA promoter, which combines the stabilized aqueous droplet networks with the LA-DNA-made LA synthetic tissues. By making use of the expression system under the control of the LA-DNA promoter, within the synthetic cells the α -hemolysin (α -HL) pore was created (Fig. 8.9B).

Once the 3D printing is done the synthetic tissue has no function; α -HL was synthesized after irradiation process, which forms pores in the droplet interface bilayer, through which electric current could travel. By patterning the synthetic cells that contained the LA-DNA, or using irradiation process, an extended 3D pathway of cells producing α -HL was generated within the synthetic tissues. This in turn gave rise to LA directional electric communication realized with external electrodes. These synthetic tissues have a great potential for use in drug delivery and tissue replacement surgeries. Owing to the multicompartamental nature of these materials, release of binary agents is possible; components (such as an enzyme and a substrate) from the agents are combined at a desired site to generate potent effectors. Synthetically tissues may also be used to replace damaged tissues, particularly for providing a provisional electric connect after a nerve damage.

Also, questions of immunogenicity and uncontrolled proliferation may be decreased with synthetic tissues when compared with therapies based on living cells. 3D fabricated scaffolds by capitalizing on the natural characteristics of tissues and organs contribute to the restoration of their original functions. 3D printing of shape-changing materials combines the merits of additive manufacturing and smart materials, which encourages the advancement of personalized medical devices.

The shape memory effect of the fabricated scaffolds allows them to deploy autonomously in inaccessible places. A shape memory tracheal stent was designed on the basis of anatomic data. A methacrylated PCL precursor, with a molecular weight of 10,000 g/mol, was being used as the bioink.⁶⁵ The printed stent can be deformed to its temporary shape, plugged in to the body, and then restored back to its permanent shape by increasing the temperature. This categorizes the scaffold as a minimally invasive device. The development of highly biocompatible materials for 4D printing, which are also smart materials, is becoming an active research area. Scaffolds capable of supporting the growth of multipotent human bone marrow mesenchymal stem cells (hMSCs) were 3D STL printed with soybean oil epoxidized acrylate.^{66,67} Laser frequency and printing speeds have great effects on the superficial structures of the polymerized soybean oil epoxidized acrylate (Fig. 8.10A). These scaffolds can regain their original shape at human body temperature (37.8°C) from a temporary shape set at 188°C.

This material showed higher hMSC adhesion and proliferation than polyethylene glycol diacrylate and had no statistical difference from Poly lactic acid (PLA) and PCL. The growth of hMSCs on the printed scaffolds is shown in Fig. 8.10B. Owing to the multipotent nature of hMSCs, these printed scaffolds have many applications for osteochondral and neural tissue engineering. Graded, complex, porous structures were designed by using castor oil-based polymers (Fig. 8.10C).^{66,67} With PCL as the control, hMSC adhesion, proliferation, and differentiation greatly increased on the polymers (Fig. 8.10D).^{66,67} Plant oil-based polymers will take the study of biobased polymers for medical applications to a new level.⁶⁸ 3D printed objects that show a designed shape change over time under tissue growth and restoration conditions were created by PCL to treat infants with severe tracheobronchomalacia.³⁵ The printed supporting structure had a customized design for patients less than 1 year old. It accommodates changes in airway size by expanding as the patient grows over 3 years (Fig. 8.11A). The material degraded completely after 3 years (Fig. 8.11B) when the matured airways were able to

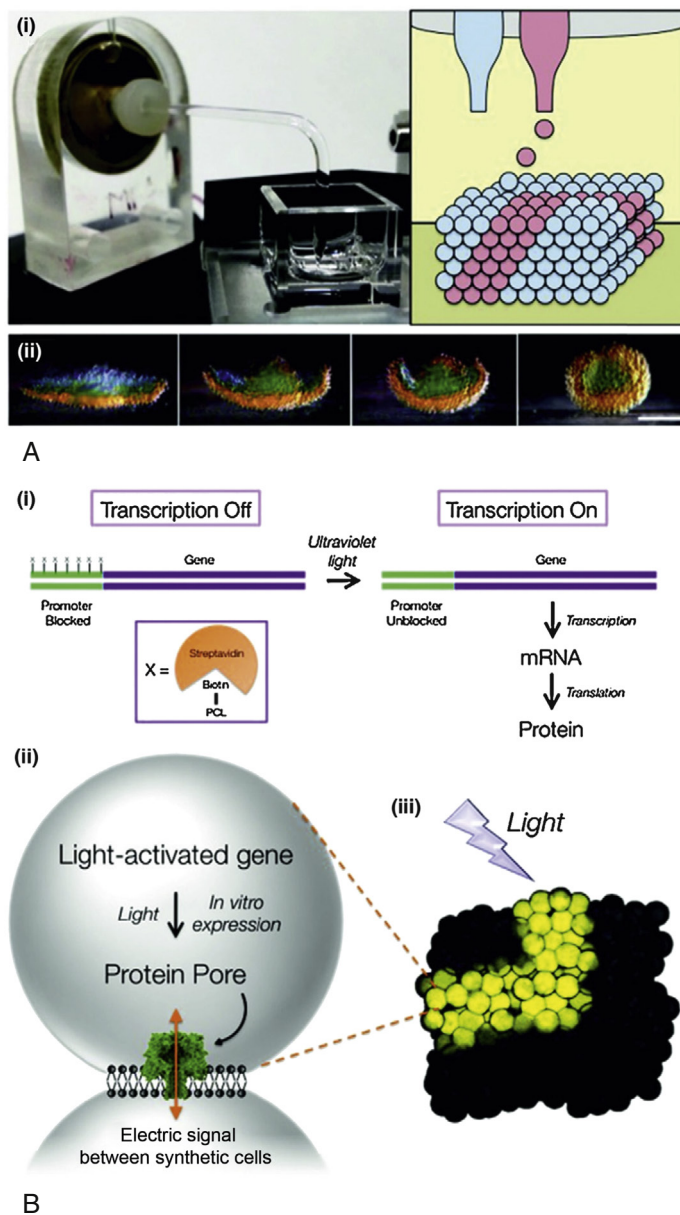


FIG. 8.9 Application of 4D printing in synthetic tissues. **(A)** (i) The 3D droplet printer. (ii) A printed four-petaled structure folded into a hollow sphere. Scale bar, 200 mm. **(B)** Light-activated synthetic tissues. (i) The light-activated DNA carries the small molecule biotin attached to a photocleavable linker (PCL) at several sites on the promoter region of a gene of interest. Biotin binds strongly to the large protein streptavidin, which blocks transcription of the gene by RNA polymerase. Low-energy ultraviolet light cleaves the PCL groups, removing the steric block. The DNA can then be transcribed into messenger RNA (mRNA) and translated to protein. (ii) A light-activated gene, encoding a protein pore, is expressed inside the synthetic cells by using an in vitro expression system. The resulting protein pores form holes in the lipid membrane between the synthetic cells, allowing electric communication. (iii) Synthetic cells can be printed into patterned synthetic tissues, which exhibit directional signaling through defined pathways, shown here by the expression of a fluorescent protein. (Adapted with permission from Villar G, Graham AD, Bayley H. A tissue-like printed material. *Science*. 2013;340 (6128):48–52. Villar G, Heron AJ, Bayley H. Formation of droplet networks that function in aqueous environments. *Nat Nanotechnol*. 2011;6 (12):803–808. Booth MJ, et al. Light-activated communication in synthetic tissues. *Sci Adv*. 2016;2 (4):e1600056.)

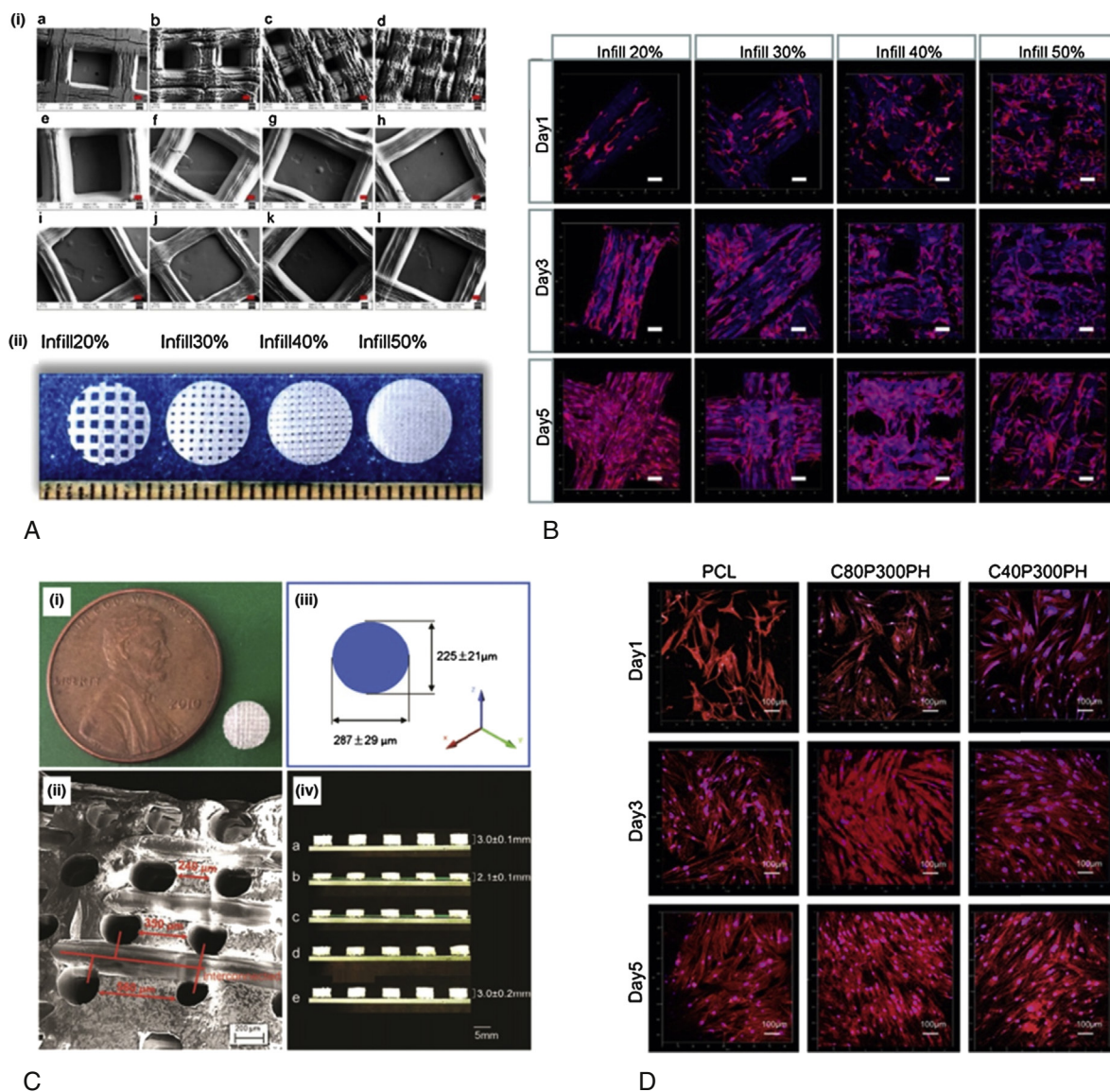
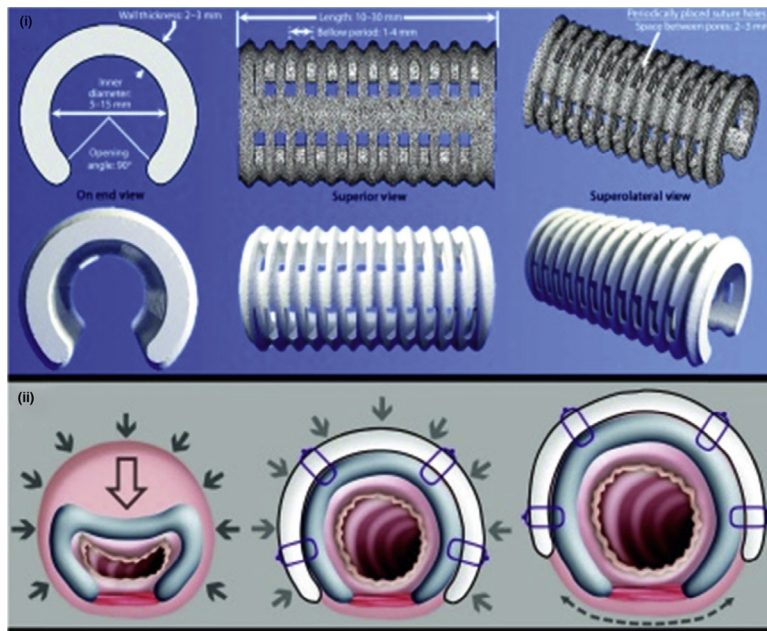
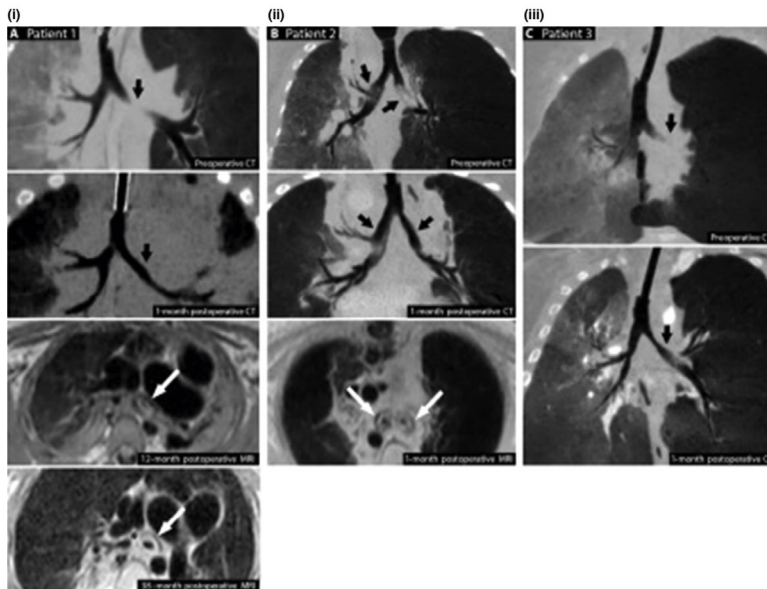


FIG. 8.10 Shape memory biomedical scaffolds. **(A)** (i) Scanning electron microscopic (SEM) images of printed scaffolds; red scale bar, 100 μm . (ii) The printed scaffolds. **(B)** Confocal microscopic images of human bone marrow mesenchymal stem cells (hMSCs) spreading on printed scaffolds. Scale bars are 100 μm . **(C)** The fabricated scaffold. (i) A 5-mm-diameter and 3-mm thickness scaffold compared with a penny. (ii) The SEM image of the pore distribution in the scaffold. (iii) Varied pore diameters in different directions. (iv) The potential for minimally invasive application. **(D)** Confocal microscopic images of hMSC growth and spreading morphology when compared with PCL control after 1-, 3-, and 5-day cultures. Red represents the cell cytoskeleton and blue represents the cell nuclei. (Adapted with permission from Miao S, et al. 4D printing smart biomedical scaffolds with novel soybean oil epoxidized acrylate. *Sci Rep.* 2016;6:27226.)



A



B

FIG. 8.11 (A) Computational image-based design of 3D printed tracheobronchial splints. **(B)** Pre- and postoperative imaging of patients. (i) Preoperative (top) and 1-month postoperative (upper middle) CT images of patient 1. Postoperative MRI (lower middle) demonstrated the presence of splint around left bronchus (arrows) in patient 1 at 12 months, and focal fragmentation of the splint due to degradation at 38 months (bottom). (ii) Preoperative (top) and 1-month postoperative (middle) CT images of patient 2. Postoperative MRI (bottom) demonstrated the presence of splints around the left and right bronchi in patient 2 at 1 month. (iii) Preoperative (top) and 1-month postoperative (bottom) CT images of patient 3. (Adapted with permission from Morrison RJ, et al. Mitigation of tracheobronchomalacia with 3D-printed personalized medical devices in pediatric patients. *Sci Transl Med.* 2015;7 (285):285ra64.)

function unaided. It has been observed that the degradation and expansion of the printed PCL device coincides with the growth of the tracheobronchial tree over time, which is called the 4D effect.

This study implies the great application potential of 4D devices, which can adapt to the growing as well as changing tissues, especially for pediatric applications.

CHALLENGES IN 4D AND 5D PRINTING

Table 8.2 lists many of the challenges facing the 3D bioprinting field, related to specific technical, material, and cellular aspects. Even though 3D bioprinting

is in its early stage, it has successfully succeeded in creating some tissues at human scale, which are approaching the functionality required for transplantation (Fig. 8.12). Technological challenges require the need for increased resolution, speed and compatibility with biologically relevant materials. As we shift from modification of preexisting technology to designing 3D bioprinters to handle specific biological components, we can extend the range of compatible materials and methods, such as deposition of materials and cells, with increasing precision and specificity. To manufacture constructs of clinically relevant sizes the speed of fabrication must be increased. We

TABLE 8.2
A Few Specific Areas Where Further Research Is Needed

Issues to be Addressed	
Area	Focus for future research
Bioprinter technology	Compatible with physiologically relevant materials and cells
	Increased resolution and speed
	Scale-up for commercial applications
	Combining bioprinter technologies to overcome technical challenges
Biomaterials	Complex combinations or gradients to achieve the desired functional, mechanical, and supportive properties
	Modified or designed to facilitate bioprinter deposition, while also exhibiting desired postprinting properties
	Use of decellularized tissue-specific ECM scaffolds to study ECM compositions and/or as printable material
Cell sources	Well-characterized and reproducible source of cells is required
	Combinations of cell phenotypes with specific functions
	Greater understanding of the heterogeneous cell types present in the tissues is required
	Direct control over cell proliferation and differentiation with small molecules or other factors
Vascularization	Well-developed vascular tree required for large tissues
	May have to be engineered in the bioprinted construct
	Capillaries and microvessels required for tissue perfusion
	Suitable mechanical properties for physiologic pressures and for surgical connection
Innervation	Innervation is required for normal tissue function
	May be inducible after transplantation using pharmacologic or growth factor signaling
	Simulation before transplantation could be achieved using bioreactors
Maturation	Time required for assembly and maturation
	Bioreactors may be used to maintain tissues in vitro
	Provide maturation factors as well as physiologic stressors
	Potential for preimplantation testing of constructs

ECM, extracellular matrix.

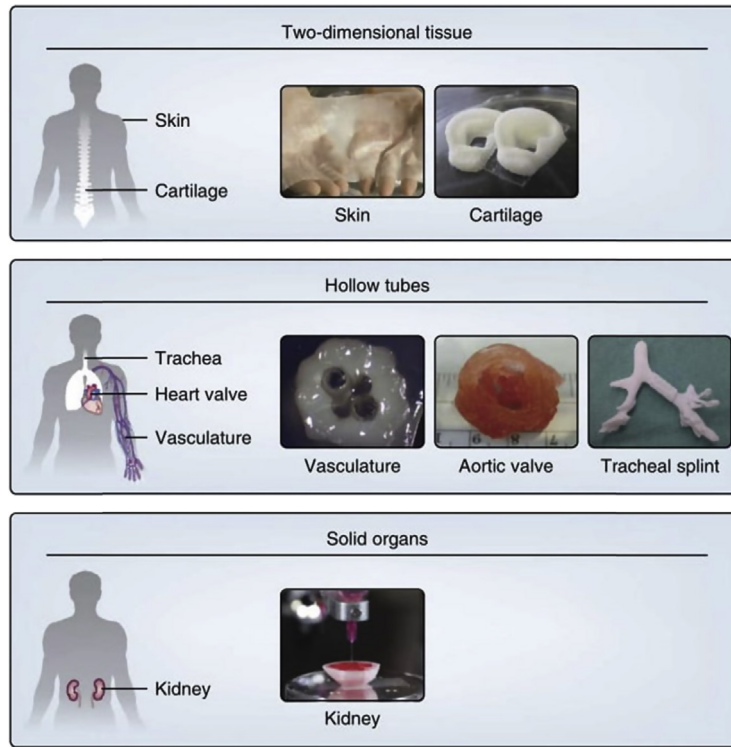


FIG. 8.12 Examples of human-scale bioprinted tissues. Skin (unpublished; Wake Forest Institute for Regenerative Medicine) and cartilage⁷⁰ substitutes developed using inkjet bioprinting systems, which are capable of fabricating tissues either in vitro or in situ. A vascular graft construct was manufactured using microextruded and fused cellular vascular rods⁷¹ and a microextrusion-bioprinted aortic valve was fabricated with dual cell types, aortic root sinus smooth muscle cells, and aortic valve leaflet interstitial cells.⁷² A laser bioprinted bioresorbable airway splint¹¹ and an early stage kidney prototype was manufactured using microextrusion bioprinting (unpublished; Wake Forest Institute for Regenerative Medicine). All these bioprinted tissues required integration of multiple components for the fabrication of functional, appropriately sized tissue constructs. (Vasculature image is reprinted from Marga F, et al. Toward engineering functional organ modules by additive manufacturing. *Biofabrication*. 2012;4:022001 with permission; aortic valve image is reprinted from Duan B, Hockaday LA, Kang KH, Butcher JT. 3D bioprinting of heterogeneous aortic valve conduits with alginate/gelatin hydrogels. *J Biomed Mater Res*. 2013;101:1255–1264 with permission from Wiley; tracheal splint image is reprinted from Zopf DA, Hollister SJ, Nelson ME, Ohye RG, Green GE. Bioresorbable airway splint created with a three-dimensional printer. *N Engl J Med*. 2013;368:2043–2045, Massachusetts Medical Society, with permission from Massachusetts Medical Society.)

can achieve this by generating miniature functional tissue blocks that can be scaled to the clinically relevant size by making use of macroscaffolds to join the blocks. To commercialize them, we may require scalable automated robotic technologies that house each of the components of the biofabrication production line.⁶⁹ It includes not only just the bioprinting device but also the manufacture of cells, materials, and other supporting components.

For 4D bioprinting to realize its potential, advances are needed in several aspects of the technology and

in our understanding of the biology and biophysics underlying regenerative processes in vivo.

At present, the materials that are being used for printing are chosen either because of their cross-linking or excursion characteristics or because of their compatibility with cell growth and function. Therefore many published studies use a limited range of materials, including collagen, hyaluronic acid, alginate, modified copolymers, and photocured acrylates. The main physiochemical parameters that determine the printability of a hydrogel are its rheologic properties and

cross-linking mechanisms. The material should be biocompatible, should be easily manipulated by the bioprinter technology to be dispensed in complex 3D structures, and should maintain cellular viability and function, which provides structural and mechanical support to the structure. Almost all human tissues have complex combinations and gradients of ECM components. Each tissue has its own specific biological and mechanical influences. It is possible that by reproducing the biomaterial environment of the organs and tissues of our interest, many of the desired mechanical and functional properties will also be reproduced. It seems doubtful that any single material will have all the properties required to recapitulate tissue function. One approach is to develop the functionally adaptive materials that reprogram the shape, properties, or functionality when required, which relies on the external stimuli. Such materials modify their function by responding to the stimuli from the body as the organ matures, in response to physiologic cues, or following externally administered stimuli that are designed to change the tissue.⁷³

One of the approaches to improvise the understanding of material environment would be to analyze the composition and distribution of ECM proteins in decellularized tissue scaffolds. The major advancement in the field would be the ability to map, image, and reproduce complex 3D structures that are composed of biologically relevant ECM proteins. Also, decellularized tissues can be used to gain a greater understanding of physiologic ECM compositions. The ECM obtained from decellularized tissues may be used as a biomaterial in bioprinting applications. Some of the other approaches include the combination of rigid thermoplastic polymer fibers with soft hydrogel constructs.⁷⁴ Structural materials could be modified with natural or synthetic factors to affect the surrounding biological behavior. This approach may fulfill both the functional requirements of the cell and the structural requirements of the 3D printed tissues.

Bioprinting demands sources of cells that are readily available, are easy to expand in culture, are nonimmunogenic, and can reproduce all the functions of the tissue or organ system. Combinations of various mature and/or multipotent cell sources can be applied to increase the efficiency of reproducing the cell phenotypes needed for specific tissues. For example, the functional building blocks of a construct can be generated by using a stem cell population derived from a functional component of the tissue. Mesenchymal stem cells derived from the bone marrow could efficiently generate the connective tissue that forms the structural components of an organ. In

addition, the recent advances in the application of small molecules to cell culture suggest that might have more direct control over cell proliferation and differentiation in the future, with several studies stating the directed differentiation of cells using small molecules.⁷⁵⁻⁷⁷

Bioprinting has other challenges too as shared by researchers in the fields of tissue engineering and regenerative medicine. It is viable to ensure sufficient vascularization of the engineered construct for long-term viability of any bioprinted organ construct. Studies have demonstrated generation of a branched vascular tree for bioprinted tissue constructs.⁷⁸⁻⁸⁰ A challenge in this approach is how far is this process compatible with the materials and cells and the other components of the printing system. Additionally, the time required for assembly and maturation of a perfused vascular network throughout the entire tissue construct may be longer than the cell survival time. Bioreactors can contribute by maintaining the viability of tissue constructs, providing the time necessary for postprocessing tissue fusion, remodeling, and maturation. Bioreactor processing can also be used in combining the factors that promote angiogenesis and innervations⁸¹ as well as factors that can maintain or preserve cell viability.⁸² In addition, bioreactors have an essential role in maintaining microenvironmental parameters such as temperature, pH, and nutrient and gas concentrations as well as in regulating specific mechanical stimulations.⁸³ These parameters should be designed and engineered for each specific tissue type and for the developmental goal.

An alternate approach to the bioprinting-transplantation paradigm is *in vivo* bioprinting in which cells and materials are directly deposited in or on the patient. By increasing the speed and the resolution of the 3D bioprinters, this approach may become viable for the *in vivo* regeneration of tissues immediately after injury or surgery. A combined robotic surgical tool and 3D bioprinter might be able to remove and replace tissues during surgery or may also be applied to accelerate the healing of the tissues removed by the surgical intervention.

3D bioprinted tissue constructs are developed for use not only in transplantation but also in drug discovery; the analysis of chemical, biological, and toxicologic agents; and in basic research. Progressive development will lead to printing tissues with increasing complexity, beginning with 2D tissues such as skin, through hollow tubes such as blood vessels, to hollow tubes such as skin, to hollow nontubular organs such as the bladder, and finally to solid organs such as the kidney (Fig. 8.13). There is increasing need to challenges such

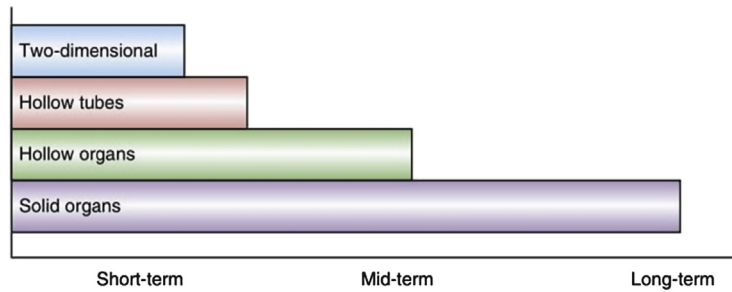


FIG. 8.13 Developments of 3D biomaterials of tissues corresponding to time. Four major types of tissues can be ranked from a simple complex: 2D tissues, such as skin; hollow tubes, such as blood vessels; hollow nontubular organs, such as the bladder; and solid organs, such as the kidney. As the complexity of tissues increases, new approaches will be needed to overcome the challenges of creating them by bioprinting. 2D organs have been fabricated and tested, and these are the first type of bioprinted tissues to be transplanted in patients. Hollow tubes, including blood vessels, trachea, and urethra, are still in their developmental phase and are likely to closely follow 2D tissues in clinical application. Hollow organs are more complex and may take longer to develop. Solid organs are the most complex and there are still many challenges to overcome in their bioprinting, especially in achieving vascularization and innervation.⁴

as including cell and material requirements, tissue maturation and functionality, and appropriate vascularization and innervation. There is need for a multidisciplinary research to meet these challenges and to realize the potential of 3D bioprinting to transform the field of regenerative medicine.

SUMMARY AND FUTURE OUTLOOK

Several 3D printing technologies have been used for 3D biofabrication, including STL and FDM. Artificial tissues and organs have been fabricated directly using different technologies such as cell culturing and/or implantation; alternatively, biological constructs encapsulating cells and biologically bioactive agents can be used in printing by using cells containing inks.⁸⁴ The currently available different forms of 4D printing are polyjet printing and syringe printing; there are several other 3D printing platforms with promising potential to contribute to 4D printing. In addition to structural design and printing techniques, ink materials are significant for achieving a 4D effect. In the 4D printing technology, 4D inks have an important role and the progressive development of 4D inks requires specific expertise and significant effort.^{85,86} Interestingly, in tissue and organ regeneration applications, the printing materials should have different unique features such as biocompatibility, which may be able to perform dynamic 4D processes in a physiologic environment. Unfortunately, most of the studies and reported materials are being used for nonbiomedical

applications, and exterior stimuli are extremely harsh for tissue and organ function. Since a long time ago, researchers have identified study areas about hydrogels, which are an important material for 4D printing.^{87,88} 4D inks have promising potential and there is no doubt about that they are required for expanding 4D printing in the near future. In summary, although 4D printing has tremendous potential and in several applications and continues to generate attention, it is still in its budding phase as a research area. Researchers are working in different areas of 4D printing, which requires several technologic advancements and progressive development in various fields including software, modeling, mechanics, and chemistry. Since several decades, self-assembling materials have been studied intensively, but only a tiny portion of these bioactive materials have been derived for 4D printing. There are multiresponsive structures that respond to different stimuli, requiring greater efforts and expertise. In addition, they have some specific requirements for use in tissue and organ regeneration applications, such as biodegradability and biocompatibility properties. 4D printing has enormous potential that may provide a novel and specific platform for biomedical and medical studies on functional synthetic tissues and organs. In 4D printing technology contains promising characteristics of 4D printing, expected plethora of unexplored research areas and disciplines with a novel wide application range of multidimensional printing, not only in the tissue and organ regeneration field but also in all areas of science and technology.

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